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Shedding light on a cryptic cavernicole: A second species of *Zenkevitchia* Birstein (Crustacea, Amphipoda, Typhlogammaridae) discovered via molecular techniques

Dmitry A. Sidorov[1], Andrey A. Gontcharov[1], Dmitry M. Palatov[2], Steven J. Taylor[3], Alexander A. Semenchenko[4]

1 *Institute of Biology and Soil Science, Far Eastern Branch of the Russian Academy of Sciences, 100-let Vladivostoku Av. 159, Vladivostok 690022, Russia* **2** *Department of Hydrobiology, Moscow State University, Leninskie Gory 1/12, Moscow 119991, Russia* **3** *Illinois Natural History Survey, University of Illinois, 1816 S. Oak St., Champaign, Illinois U.S.A.* **4** *Far Eastern Federal University, Suhanova St. 8, Vladivostok 690950, Russia*

Corresponding author: *Dmitry A. Sidorov* (biospeorossica@gmail.com)

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Abstract

The Abkhazian region, in the southern foothills of the Caucasus Mountain Range, comprises a unique natural environment containing numerous subterranean habitats with relict and endemic lineages of obligate stygofauna. We aimed to assess the molecular phylogenetic relationships of Typhlogammaridae species from Balkan and Transcaucasian caves using the mitochondrial cytochrome c oxidase I (COI) in hopes of discovering previously undetected biodiversity. Our results showed molecular divergence within the genus *Zenkevitchia* Birstein, with two distinct groups located in the karstic regions Gudauta-Sukhumi and Gulripshi, respectively. These data indicated the existence of a new species (sequence divergences between groups of >14.3%) within the hitherto monotypic genus *Zenkevitchia* and allowed us to estimate the taxonomic relationship between *Zenkevitchia admirabilis* and *Z. yakovi* **sp. n.**, based on examined morphological features and molecular phylogenetic relationships. We were unable to detect reliable morphological differences between *Z. yakovi* **sp. n.** and *Z. admirabilis*, highlighting the cryptic nature of the new species and the value of inclusion of molecular data in taxonomic studies.

Keywords

Amphipoda, *Zenkevitchia*, molecular identification, cytochrome *c* oxidase I, Transcaucasia

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Introduction

Molecular identifications based on the mutability of DNA barcode region of the cytochrome c oxidase I (COI) of mitochondrion (Hebert et al. 2003) have advanced biodiversity inventories and resulted in the discovery of many new species (e.g., Smith et al. 2007), including amphipod taxa (Bradford et al. 2010).

The Typhlogammaridae Bousfield, 1978 is the endemic Balkan-Transcaucasian family of cavernicole amphipods belonging to the huge Gammaridea suborder and represented in subterranean waters by 8 decribed species (and sub-species) in the region (Väinölä et al. 2008). The genus *Zenkevitchia* is monotypic, represented only by *Zenkevitchia admirabilis* Birstein, 1940 in Abkhazia. The species described as *Zenkevitchia revazi* Birstein & Ljovuschkin, 1970 has been transferred to the genus *Anopogammarus* (Karaman and Barnard 1979). Analyzing the geographical distribution of *Zenkevitchia admirabilis* is broadly distributed across the region, with published records across several karst areas: Gudauta region (Andreevskaya, Tarkiladze and Anakopiyskaya caves), Gulripshi district (Verkhne-, Sredne-, Nizhne-Shakuranskaya and Tsebeldinskaya caves) (Birstein 1940, 1941; Birstein and Ljovuschkin 1970), and the subterranean source of the Chernaya river near Gudauta (Derzhavin 1945). In his works, Birstein indicated that there was a wide range of morphological variability within *Z. admirabilis*. Carefully analysis of Birstein's works suggests that *Z. admirabilis* appears to be represented by a several morphotypes. In particular, the body size of adults varies widely. But characters which might indicate an excessive variability either were not specified by Birstein, or they were not clearly differentiated. The type series of *Z. admirabilis* has not been properly fixed according to the requirements of the Commission on Zoological Nomenclature in Code (1999: 75) (Article 72.2), as the type locality was not specified in the original description (Birstein 1940). In addition, the morphological and taxonomic boundaries of this species are vague, and thus the systematics of the genus *Zenkevitchia* appears to be in a state of confusion and in need of revision. Virtually nothing is known about the biology and origin of this enigmatic taxon. The heavily spinous outer plate of maxilla 1 implies a filtering function (Barnard and Barnard 1983). According to the data set and phylogenetic analysis of Hou et al. (2014) using nuclear 28S rDNA, the genus *Zenkevitchia* is sister to *Anopogammarus* Derzhavin, 1945, though this grouping has no bootstrap support.

In the present study, we sampled *Zenkevitchia* in limestone karstic caves located in the Shakuranian cave-complex near Tsabal in the Chjalta mountain range (south-central Abkhazia). We evaluate the possible existence of hidden diversity within the genus *Zenkevitchia* using the DNA barcode region of the cytochrome *c* oxidase subunit I mitochondrial gene (COI) and evaluate the morphological traits of putative morphotypes.

Material and methods

Taxonomic sampling

Samples of blind, unpigmented amphipods were collected from two adjacent (4.1 km) localities in Abkhazia. All specimens were collected a fine-mesh hand-held dip net with a mesh size of 250 μm and fixed in 96% ethanol at the field site.

Morphology

A complete dissection was made of all pertinent morphological structures of specimens in the type series of the new species and permanent slide preparations were made using polyvinyl lactophenol (PVL) and methylene blue stain. Prior to dissection, body length was recorded by holding the specimen straight and measuring the distance along the dorsal side of the body from the base of the first antennae to the base of the telson. A Lomo MBS-9 stereomicroscope with a scaled micrometer eyepiece was used to make the measurements, and appendages were drawn using a Carl Zeiss NU-2 compound microscope equipped with a drawing device as modified by Gorodkov (1961).

To better understand the structure of outer plate of maxilla 1, and to facilitate counting the number of spines, the specimens were slightly crushed and squeezed in glycerol. The term "palmar angle" of the gnathopod propodi refers to the angle formed at the end of the palm and beginning of the posterior margin or the point at which the tip of the dactylus closes on the propodus (Birstein 1941); later the same structure was also named "defining angle" (Holsinger 1974), but we believe that the Birstein's combination of words better describes this feature. The nomenclature for setal patterns on article 3 of the mandibular palp follows Karaman (1970) and Stock (1974). The species diagnosis and taxonomic comparison were generated from a DELTA database (Dallwitz 2005) for the typhlogammarid genera and species of the world.

The description is based on the type series, with the material examined deposited in the Zoological Museum of the Far East Federal University, Vladivostok (FEFU; the holotype is here) and in the research collection of the Institute of Biology and Soil Science, Vladivostok (IBSS).

DNA extraction, PCR, sequencing and analyses

Total DNA was extracted from the muscle tissue with a DNeasy Blood & Tissue kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's guideline. The COI gene fragment was amplified using the universal primers HCO2198 and LCO1490 (Folmer et al. 1994). The annealing temperature was set to 40 °C for 20 s. The PCR products were sequenced directly using the same primers and a BigDye terminator v. 3.1 sequencing kit (Applied Biosystems, USA). Sequences were analyzed

on an ABI 3130 genetic analyzer (Applied Biosystems, USA) and assembled with the Staden Package v. 1.4 (Bonfield et al. 1995).

The Akaike information criterion (AIC) in MODELGENERATOR 0.85 (Keane et al. 2006) was used to select the model of sequence evolution best fitting our data set (HKY+G). The data set was analyzed using the maximum-likelihood (ML) algorithm in SEAVIEW 4.0 (Gouy et al. 2010). A standard BioNJ initial tree was obtained automatically by applying the Nearest-Neighbor-Interchange (NNI) for tree inference. To assess support for clades 1,000 bootstrap replicates (Felsenstein 1985) were performed.

Data resources

The data underpinning the analysis reported in this paper are deposited in the Dryad Data Repository at http://dx.doi.org/10.5061/dryad.88fm6.

Results

Molecular phylogenetic analysis

Taking into account reports of a rather wide range of body sizes of adults of *Z. admirabilis* from the region, we compared partial mitochondrial COI gene sequences for evaluation of the hidden diversity within the genus. The molecular analysis comprised 10 sequences of the family Typhlogammaridae of a total 428–620 bp in length available in GenBank (NCBI KF478522, KF478584, KF478590–KF478592, KF478599, KF478600), together with 3 individuals (NCBI KP844572–KP844574) representing of the newly described species from two adjacent (4.1 km) localities in Abkhazia. Phylogenetic relationships within Typhlogammaridae (Figure 1) were assessed using a data set consisting of all described species in the family besides *Anopogammarus birsteini* Derzhavin, 1945, a species inhabiting springs on the shores of Lake Goluboe (43.35087, 40.41115) near Gagry, Abkhazia, 80 km north of our study area.

Each amphipod specimen sequenced for this study along with referred in GenBank was characterized by a unique mitochondrial COI sequence excepts for both from the Sredne-Shakuranskaya Cave which are identical. *Typhlogammarus mrazeki* with other closer unidentified congener species accessions KF478590, KF478591 formed a strongly supported clade (95%) with *Metohia carinata* branching basally (97%). Relationship between unidentified *Accubogammarus* and *Anopogammarus revazi* remained unresolved in our analysis. Analysis of the mt-DNA barcode region proved to be an effective tool for the molecular identification and detection of cryptic diversity within *Zenkevitchia* inhabiting limestone caves in Abkhazia (Table 2), where two genetically distinct groups (Figure 1) were recovered. These groups are represented by specimens of *Z. admirabilis* collected in the Gudauta-Sukhumi region (Cluster B) and, in the Gulripshi region (Cluster A), by the new species describe below. The two groups showed genetic divergences ranging from 14.3% to 19.6%

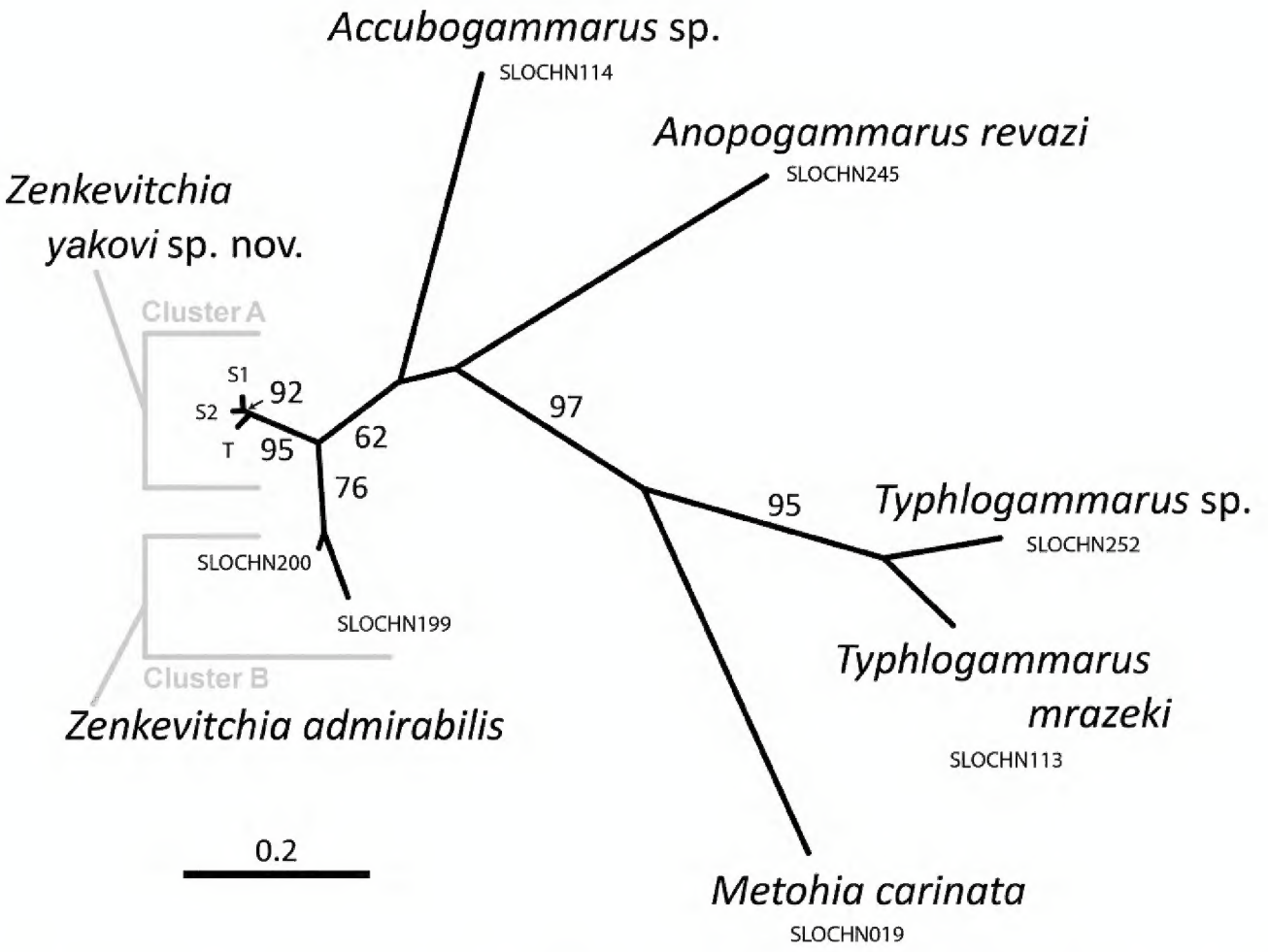

Figure 1. Unrooted maximum-likelihood cladogram with bootstrap probabilities based on the mitochondrial cytochrome c oxidase I (COI) sequences for representatives of species within the family Typhlogammaridae. Specimen labels refer to information given in Table 2. Scale bar indicates the number of substitutions per site.

based on the uncorrected pairwise distances (Table 3). The monophyly of *Zenkevitchia* within the Typhlogammaridae is supported by moderate bootstrap values (62%) and the haplotypes for the two *Zenkevitchia* species differ from the rest of the Typhlogammaridae by the uncorrected pairwise distances mean sequence divergences of 45.4%.

Descriptive part

Order Amphipoda Latreille, 1816
Superfamily Gammaroidea Leach, 1814
Family Typhlogammaridae Bousfield, 1978

Genus *Zenkevitchia* Birstein, 1940

syn.: *Zenkevitchia* Birstein, 1940: 51, fig. 4. — Birstein 1941: 260, figs 1–3. — Derzhavin 1945: 34. — Birstein and Ljovuschkin 1970: 1472, fig. 1. — Barnard and Barnard 1983: 507.

Type species. *Zenkevitchia admirabilis* Birstein, 1940, by monotypy.

***Zenkevitchia yakovi* Sidorov, sp. n.**
http://zoobank.org/44C8C4D2-F34C-4F02-864A-08FE8D490B91
Figures 2–7

Diagnosis (both sexes). Robust, small-sized species of gammarid-like habitus (sexual dimorphism marked, i.e., some males larger than females, gnathopods 1–2 and uropod 3 sexually dimorphic). Antenna 1 short, reaching 50% length of body; antenna 2 short, reaching about 40–60% length of antenna 1, gland cone long, reaching half of peduncle segment 4. Maxilla 1 inner plate triangular with 20–24 plumose setae, outer plate of filtration-type, with 105–114 multi-toothed sickle-shaped comb-like spines; palps symmetrical and reduced. Maxilla 2 inner and outer plates apically with numerous setae in two rows. Mandibular palp article 3 with 1 A group of 2 setae, 2 B setae, 13 D setae, 4–5 E setae. Gnathopods 1–2 small, with propodus not larger than corresponding coxa; palmar angles of both gnathopods with singly notched, strong corner spine on inner face; dactyli with 1 seta along outer margin, 1 stiff seta at nail base along inner margin, nails short; inner margin of dactyli of pereopods 3–7 with 1 stiff seta, with 1 thin seta at nail base. Urosomites 1–2 with dorsolateral groups of spines. Pleopods 1–3 with 2 coupling setae (retinacula) each accompanied with 1 strong, serrate spine (2+1). Uropod 1 rami almost equal, inner ramus scarcely shorter than outer ramus. Uropod 3 short, as long as uropod 2, endopodite small, 0.4–0.5× shorter than

Table 1. Variation in morphological characters in adult specimens of *Zenkevitchia yakovi* sp. n. and comparison with published data for *Z. admirabilis*.

Character	Z. yakovi sp. n.	Z. admirabilis Birstein, 1940	Z. admirabilis (Birstein, 1941)	Z. admirabilis (Birstein & Ljovuschkin, 1970)
Body length, mm	8.0–9.75 (♀♀) 5.0–10.0 (♂♂)	– 14.0 (♂♂)	4.0 (♀♀) –	up to 20.0 (sex not specified)
Antenna 1, primary flagellum	27 articles (♂♂) 19–20 articles (♀♀)	31 articles	19-24 articles	38 articles
Antenna 2, primary flagellum	11 articles (♂♂) 9 articles (♀♀)	16 articles	8 articles	17 articles
Maxilla 1, palp	5–8 spines	up to 10 spines	5 spines	~ 8 spines
Maxilla 1, inner plate	20–24 setae	- ? -	15-17 setae	~ 35 setae
Maxilla 1, outer plate	105–114 spines	- ? -	23 spines	>50 spines
Gnathopod 1, male p.m.[1]	9 spines	6-7 spines	- ? -	9-22 spines
Gnathopod 1, female p.m.	7 spines	- ? -	4 spines	
Gnathopod 2, male p.m.	6 spines	6-7 spines	- ? -	10-13 spines
Gnathopod 2, female p.m.	5 spines	- ? -	4 spines	
Origin of material	Gulripshi: "Istočnik Tcebel'da" and Sredne-Shakuranskaya caves	not specified	Sukhumi: small cave near Andreevka (Apra)	not specified

Note : [1]p.m. – palmar margin.

Table 2. List of the specimens, sampling sites and accession numbers of the sequences for COI included in this study. References are given for sequences obtained from GenBank.

Specimen	Locality	GenBank acc. no.	Reference / or year of sampling
Zenkevitchia yakovi T	Georgia, Abkhazia: "Istočnik Tcebel'da" cave, 43.026216 N 41.283022 E	KP844572	This study / 2012
Zenkevitchia yakovi S1	Georgia, Abkhazia: Sredne-Shakuranskaya cave, 43.029748 N 41.333198 E	KP844573	This study / 2012
Zenkevitchia yakovi S2	Georgia, Abkhazia: Sredne-Shakuranskaya cave, 43.029748 N 41.333198 E	KP844574	This study / 2012
From GenBank			
Accubogammarus sp. SLOCHN114	Montenegro: Grahovo, Vojvode Dakovića cave	KF478592	Hou et al. (2014)
Anopogammarus revazi SLOCHN245	Georgia: Martvili, cave Motena	KF478522	Hou et al. (2014)
Metohia carinata SLOCHN019	Montenegro: Rijeka Crnojevića, Obodska cave	KF478584	Hou et al. (2014)
Typhlogammarus mrazeki SLOCHN113	Montenegro: Cetinje, Lipska cave	KF478590	Hou et al. (2014)
Typhlogammarus sp. SLOCHN252	Croatia: Zrmanja, Krupa cave	KF478591	Hou et al. (2014)
Zenkevitchia admirabilis SLOCHN199	Georgia, Abkhazia: Sukhumi, Verhnie Pešeri, Verhne-esherskaja (=Sobachya) cave	KF478600	Hou et al. (2014)
Zenkevitchia admirabilis SLOCHN200	Georgia, Abkhazia: Gudauta, Lihni, Tarkili (=Tarkiladze) cave	KF478599	Hou et al. (2014)

Table 3. Estimates of pairwise sequence divergence (uncorrected *p*-distances) of partial mitochondrial COI gene among species and haplotypes of the Balkan and Caucasian Typhlogammaridae.

	1	2	3	4	5	6	7	8	9
1. *Zenkevitchia yakovi* S1	—								
2. *Z. yakovi* S2	0.0	—							
3. *Z. yakovi* T	0.005	0.005	—						
4. *Z. admirabilis* SLOCHN199	0.196	0.196	0.196	—					
5. *Z. admirabilis* SLOCHN200	0.143	0.143	0.143	0.089	—				
6. *Accubogammarus* sp. SLOCHN114	0.300	0.300	0.300	0.343	0.306	—			
7. *Typhlogammarus* sp. SLOCHN252	0.491	0.491	0.478	0.474	0.521	0.554	—		
8. *T. mrazeki* SLOCHN113	0.478	0.478	0.466	0.414	0.450	0.484	0.137	—	
9. *Metohia carinata* SLOCHN019	0.513	0.513	0.500	0.532	0.547	0.481	0.460	0.503	—
10. *Anopogammarus revazi* SLOCHN245	0.379	0.379	0.385	0.349	0.359	0.465	0.471	0.457	0.603

exopodite. Telson with 1 or 2 distal spines per lobe. Coxal gills 2–7 stalked, triangular or sacciforme, largest on gnathopod 2, successively smaller on pereiopods 3 to 7, gill 7 the smallest. Body length 8.0–9.75 mm (females), 5.0–10.0 (males).

Type material. Holotype: male, 10.0 mm, X43382/Cr-1613-FEFU, completely dissected and mounted on a single glass slide. Abkhazia, Gulripshi district, Tsabal,

Figure 2. Photograph of live specimen of *Zenkevitchia yakovi* sp. n. in the cave "Istočnik Tcebel'da", from right side. Photography by A. Korotaev.

"Istočnik Tcebel'da" Cave, 43.026216, 41.283022, cave pool, 31.01.2012, coll. D.M. Palatov. Paratypes: X43383/Cr-1614-FEFU, 1 male, 8.0 mm, 1 female, 8.5 mm (oostegites weakly differentiated), with same data as holotype.

Additional material examined (not placed in the type series). All specimens measured, partially dissected and stored in vial (1-11/1sd-IBSS), 4 females with oostegites not well differentiated (2x8.0 mm, 8.5 mm, 9.75 mm), 9 males (3x8.0 mm, 4x8.5 mm, 9.75 mm, 5.0 mm), 1 juvenile (3.0 mm); with same data as holotype; ~ 4.1 km E of "Istočnik Tcebel'da" Cave, Sredne-Shakuranskaya Cave, 43.029748, 41.333198, small cave river, 30.01.2012, coll. D.M. Palatov.

Etymology. The specific epithet *yakovi* (Latin) refers to the first name of Yakov Avadievich Birstein a famous zoologist is known for his outstanding contributions to the systematics of subterranean crustaceans.

Description. *Male*, 10.0 mm (X43382/Cr-1613-FEFU). **General body morphology** (Figures 2, 3H, 4A, H, 7E). Body smooth, lacking dorsal cuticular elements (keel or tubercles) on pereon and pleon. *Head* as long as first pereon segment; lack rostrum; inferior antennal sinus shallow, sub-rounded. Eyes absent. Pereonite 7, pleonites 1–3 and uronite 1 with median and lateral groups of thin setae. Urosomites 1 and 2 on dorsal surface with lateral groups of spines accompanied with setae. *Epimeral plate 1*: postero-ventral corner acuminate; posterior and ventral margins convex; 1 stiff setae along ventral margin, 3 setae along posterior margin. *Epimeral plate 2*: postero-ventral corner acuminate; posterior margin straight; ventral margin convex; 3 notched spines

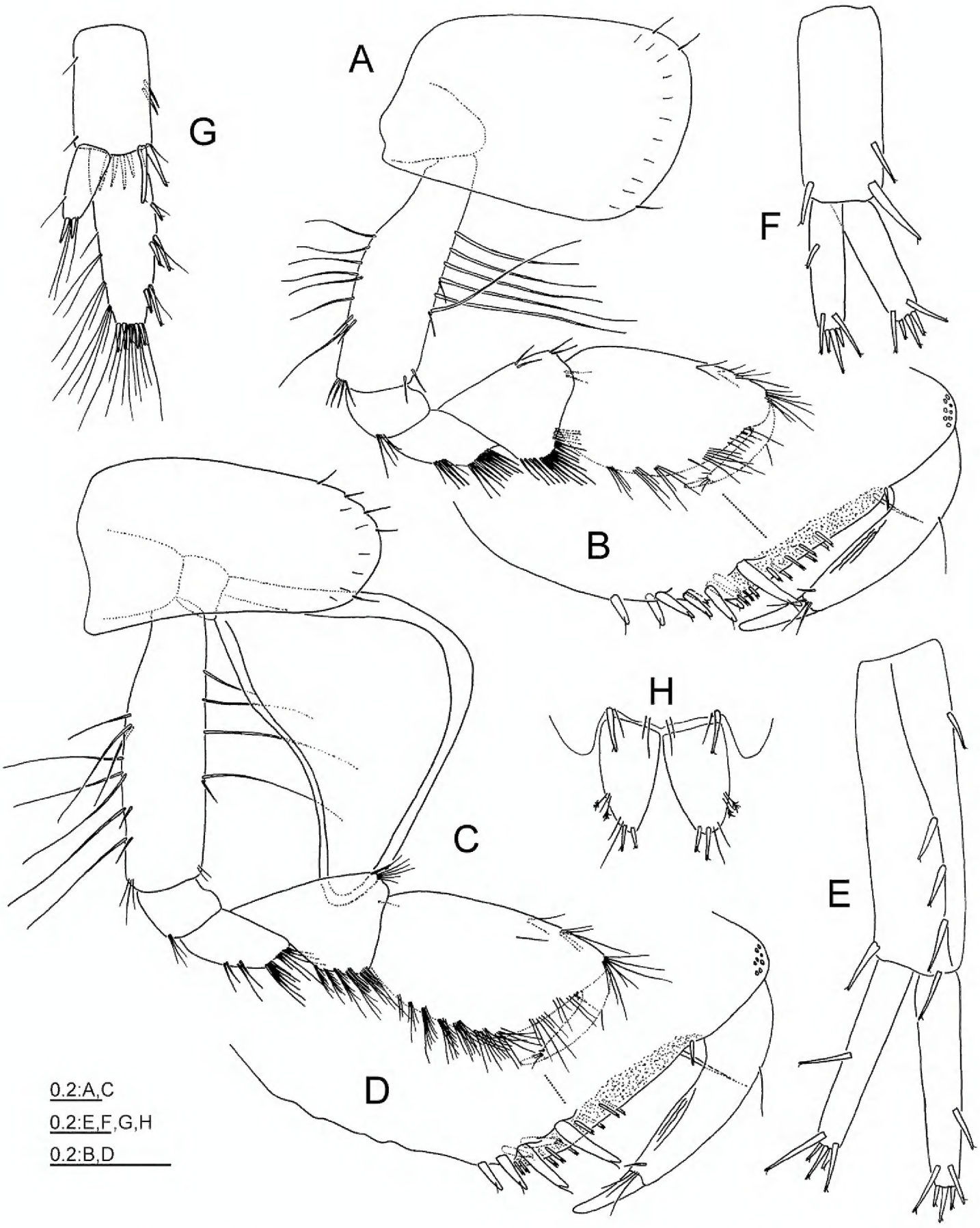

Figure 3. *Zenkevitchia yakovi* sp. n.: holotype, male (10.0 mm), X43382/Cr-1613-FEFU. **A** Gnathopod 1, lateral view **B** Enlarged palmar part of gnathopod 1 **C** Gnathopod 2, lateral view **D** Enlarged palmar part of gnathopod 2 **E, F, G** Uropods 1–3, dorsal views **H** Telson, dorsal view.

along ventral margin, 4 setae along posterior margin. *Epimeral plate 3*: postero-ventral corner acuminate; posterior margin slightly concave; ventral margin convex; 4 notched spines along ventral margin, 3 setae along posterior margin. *Telson* width : length ratio is 1 : 0.85; cleft 0.9 of length; only 2 sub-apical spines per lobe present, these

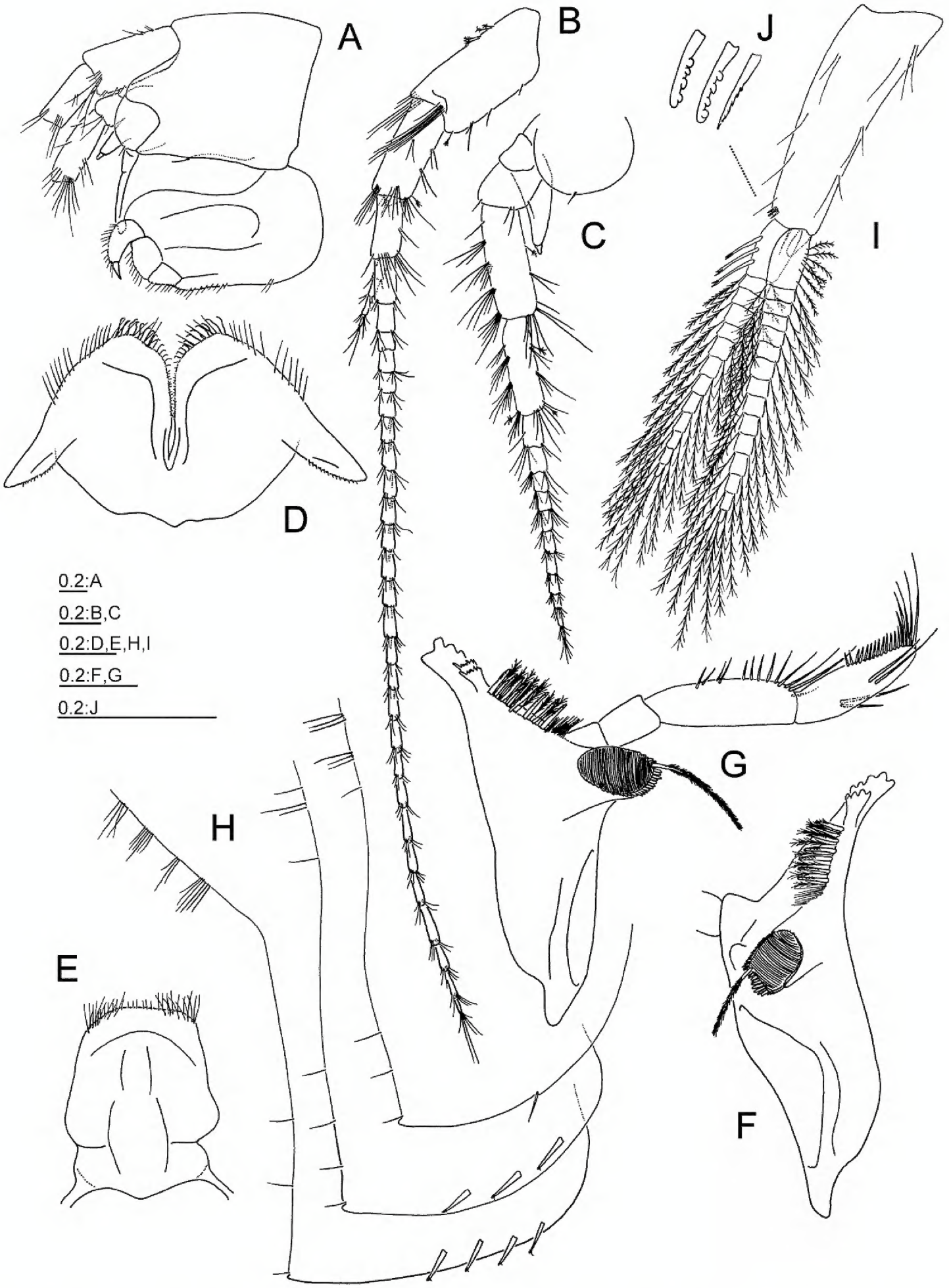

Figure 4. *Zenkevitchia yakovi* sp. n.: holotype, male (10.0 mm), X43382/Cr-1613-FEFU. **A** Head, lateral view **B, C** Antenna 1 and 2, lateral views **D** Labium, ventral view **E** Labrum, anterior view **F, G** Left and right mandibles, medial views **H** Epimeral plates 1–3, lateral views **I** pleopod 2, medial view **J** Coupling setae (retinacula), medial view.

are 0.25–0.30× telson length, each accompanied by 1 or 2 plumose seta. **Antennae** (Figures 2, 4B, C). *Antenna 1* 0.5× of body length; flagellum with up to 27 articles; each article with 5–6 short setae; peduncular articles in ratio 1 : 0.8 : 0.5; proximal article of peduncle distally with 2 medial sets of long setae; accessory flagellum 4-articulated. Length ratio antenna 1 : 2 as 1 : 0.57; flagellum of *antenna 2* with 11 articles, each article densely setose; peduncle articles lengths 4 : 5 is 1 : 0.8; flagellum slightly longer than peduncle (articles 4+5); peduncular articles 4 and 5 with sets of long stiff setae laterally; gland cone long, reaching half of peduncle segment 4. **Mouth parts**, typical gammarid except for unusual filtration-type maxilla 1 (Figures 4D–G, 5A–H). *Labrum* rounded, clypeus detached, longer than broad. Inner lobes of *labium* absent, outer lobes broad with stiff curved setae marginally, mandibular process distinct (narrow). *Left mandible*: incisor with 5 teeth, lacinia mobilis with 4 teeth; between lacinia and molar row of 7 serrate spines. *Right mandible*: incisor process with 4 teeth, lacinia mobilis bifurcate with several small denticles, between lacinia and molar a row of 8 serrate spines; triturative molar process with long lanose seta. *Mandibular palp* article 2 slightly longer than article 3 (distal); proximal palp article without setae; the second article with 13 setae; distal article narrowed, with 1 A group of 2 setae, 2 B setae, 13 D setae, 4–5 E setae. *Maxilla 1* palp reduced, distal article with 5 or 6 apical strong serrate setae (both palps symmetrical); outer plate with 114 multi-toothed sickle-shaped spines; inner plate triangular with 24 plumose setae. *Maxilla 2* inner plate smaller than outer one with oblique row of 24 plumose setae; both of them apically with numerous setae in two rows. *Maxilliped* palp article 2 with about 70 setae along inner margin; article 4 (distal) with dorsal seta, bearing 5 seta at the nail base, nail shorter than pedestal; outer plate with 15 flattened spines and 9 long plumose setae on apex; inner plate with 3 strong spines and 5 stiff naked setae on apex, 26 plumose setae on ventral face, 23 stiff denticulate setae in 3 rows on dorsal face. **Coxal plates, gills** (Figures 3A, C, 6A, C, E, G, I). *Coxal plate 1* of rectangular shape, antero-ventral margin extended with 3 setae. *Coxal plate 2* of rectangular shape, antero-ventral margin narrowed with 5 setae. *Coxal plate 3* width : depth is 0.5 : 1; along antero-ventral margin 4 setae. *Coxal plate 4* of sub-quadrate shape, width : depth is 0.9 : 1; posteriorly with prominent excavation; along ventral margin 10 setae. *Coxal plates 5–7* progressively smaller towards the posterior; *coxal plates 5–6*: only anterior lobe well-developed; posterior margin simple or pointed with 1 seta. *Coxal plate 7* semicircular, along posterior margin 3 setae in shallow serration. *Coxal gills 2–7* stalked, large but progressively smaller towards the posterior; gills 2–4 triangular, gills 5–7 saccular or irregularly ovoid. **Gnathopods 1 and 2** (Figures 3A–D). *Gnathopod 1*, ischium with postero-distal set of setae. Carpus 0.4× length of basis and 0.57× length of propodus; anterior margin of carpus with two group of setae; carpus posteriorly with transverse row of lateral and sub-marginal setae. Propodus pyriform, palm straight with cutting margin acanthaceous and shorter than posterior margin; along posterior margin 3 sets of simple setae; anterior margin with 3 pairs of setae, antero-distal group with 7 setae; palmar margin with short, notched setae along outer and inner faces and armed with row of 4 distally notched, robust spines on inside and 5 on outside, palmar angle with 1 strong corner spine on inner

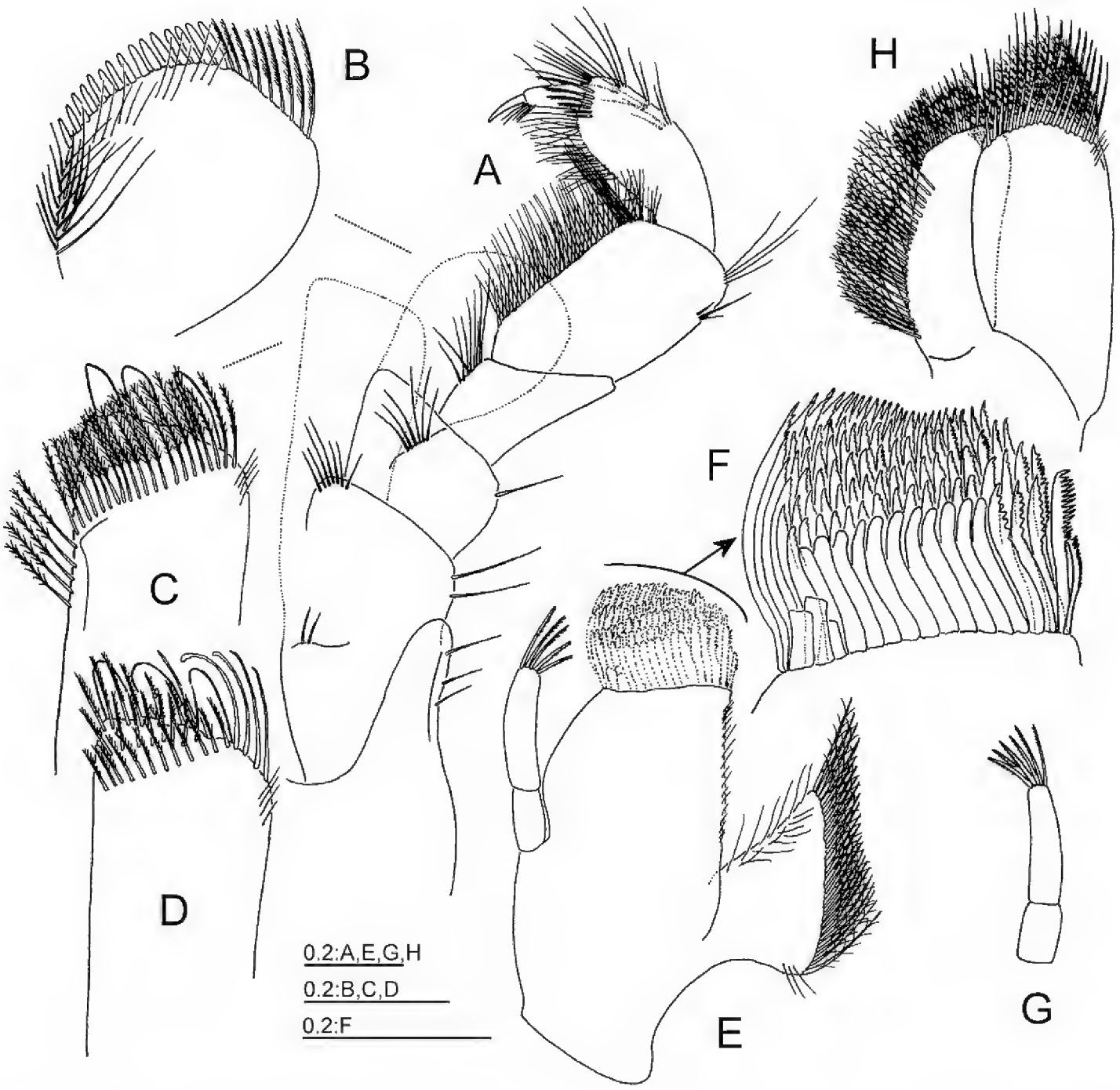

Figure 5. *Zenkevitchia yakovi* sp. n.: holotype, male (10.0 mm), X43382/Cr-1613-FEFU. **A** Maxilliped, ventral view **B, C** Enlarged outer and inner plates of maxilliped, ventral view **D** Enlarged inner plate of maxilliped, dorsal view **E** Left maxilla 1, dorsal view **F** Enlarged outer plate of maxilla 1 **G** Palp of right maxilla 1, dorsal view **H** Maxilla 2, dorsal view.

face; nail 0.3× total length of dactylus, a 1 seta along anterior margin, 1 short stiff seta along inner margin and 3 setules at hinge. *Gnathopod 2*, basis width : length is 0.29 : 1. Ischium with 4 sets of postero-distal setae. Carpus 0.35× length of basis and 0.5× length of propodus; anterior margin of carpus with 1 distal set of setae; carpus posteriorly with 5 lateral sets of setae. Propodus small (compared to the body) and 1.14× larger than propodus of gnathopod 1; propodus sub-quadrate, palm straight with cutting margin acanthaceous and shorter than posterior margin; posterior margin with 7 rows of simple setae; anterior margin with 2 sets of setae; antero-distal group with 8 setae; palmar margin with short, notched setae along outer and inner faces and armed with row of 4 distally-notched robust spines on inside and 2 on outside, palmar angle with 1 strong corner spine on inner face; dactylus similar to that of gnathopod

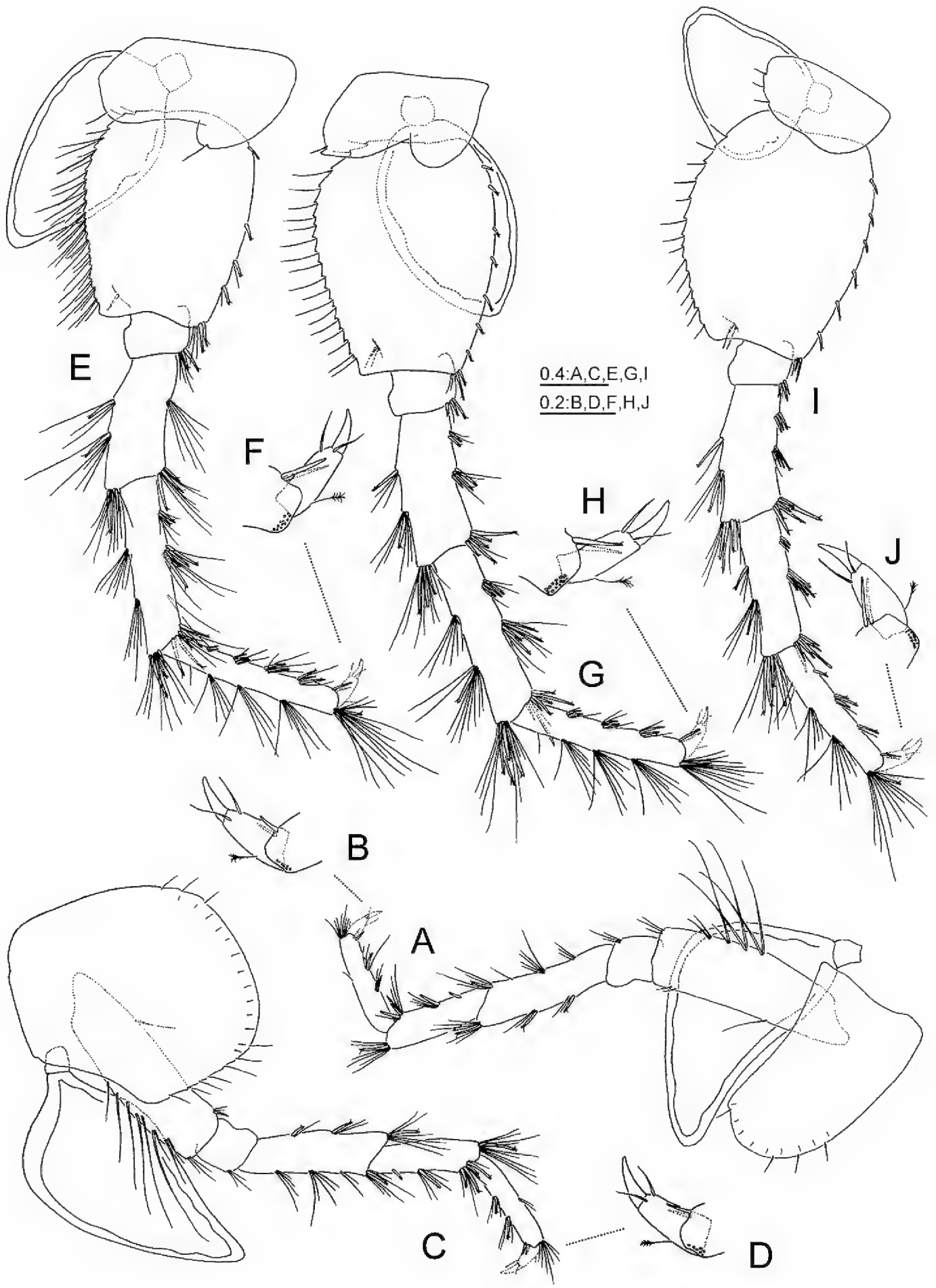

Figure 6. *Zenkevitchia yakovi* sp. n.: holotype, male (10.0 mm), X43382/Cr-1613-FEFU. **A, C, E, G, I** Pereopods 3–7, lateral views **B, D, F, H, J** Enlarged dactyli of pereopods 3–7, lateral views.

1. **Pereopods 3–7** (Figures 6A–J). Lengths of *pereopods 3–4* equal. Dactylus 4 0.4× propodus 4; nail length 0.4× total dactylus length. Dactyli 3–4 with dorsal plumose seta; inner margin with 1 stiff seta and 1 thin seta at hinge. Lengths of pereopods 5 : 6

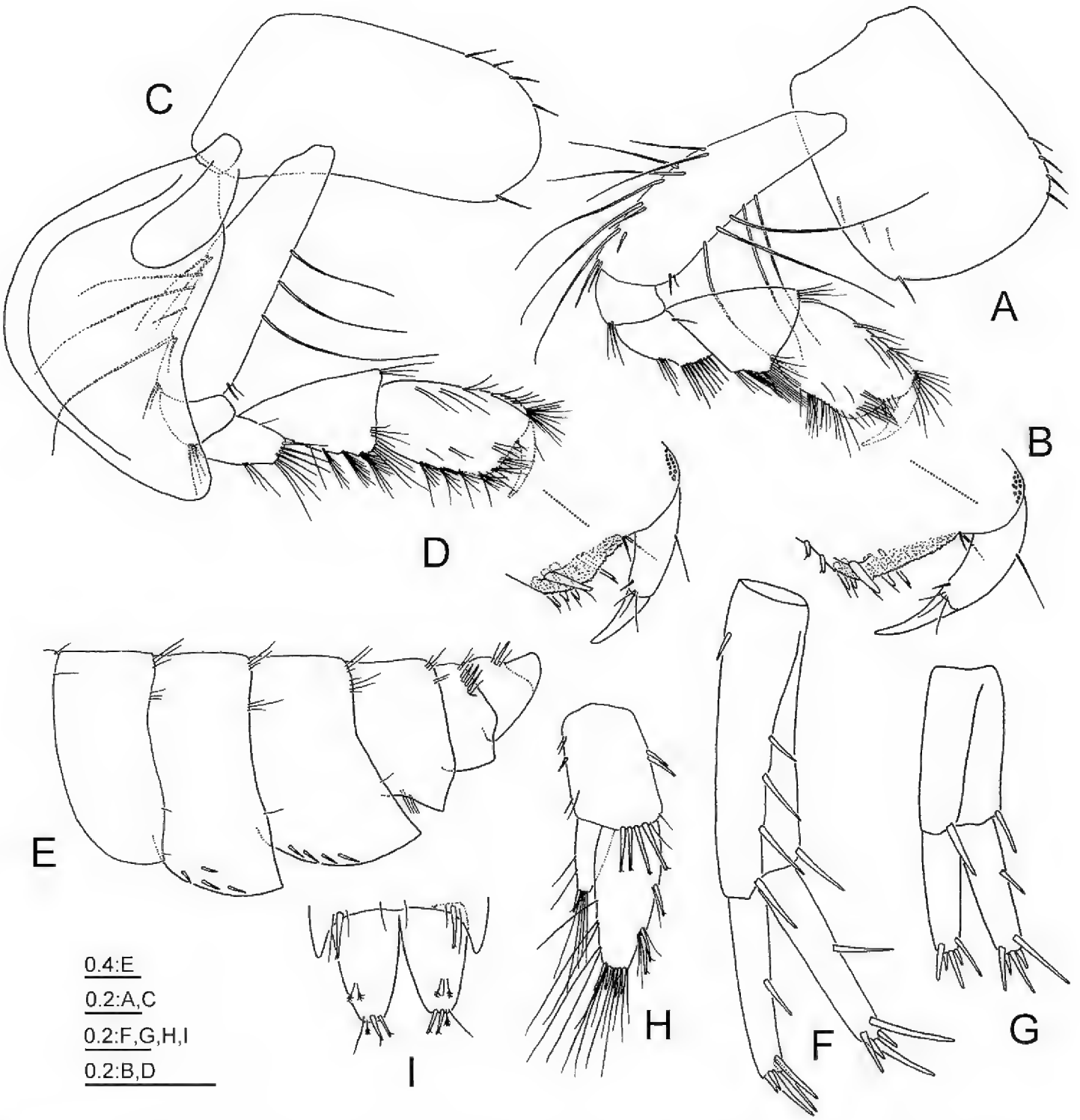

Figure 7. *Zenkevitchia yakovi* sp. n.: paratype, female (8.5 mm), X43383/Cr-1614-FEFU. **A** Gnathopod 1, medial view **B** Enlarged palmar part of gnathopod 1 **C** Gnathopod 2, medial view **D** Enlarged palmar part of gnathopod 2 **E** Metasome and urosome, lateral view **F, G** Uropods 1 and 2, dorsal views **H** Uropod 3, ventral view **I** Telson, dorsal view.

: 7 is 1 : 1.1 : 0.9. *Pereopod 7* length 0.45× body length. Bases 5–7 narrowed distally, length : width is 1 : 0.77–0.92; posterior margin convex (distinctly in basis 7), without postero-distal lobes; posteriorly marginal serrations with long setae (expressed in basis 5); anteriorly 5–7 notched spines. Dactylus 7 length 0.3× propodus 7 length. Dactyli 5–7 with dorsal plumose seta; inner margin with 1 stiff seta and 1 thin seta at hinge. **Pleopods and uropods** (Figures 2, 3E–G, 4I, J). *Pleopods 1–3* sub-equal, each with 2 coupling setae accompanied by 1 strong, serrate spine; peduncular articles fringed with long, thin setae; proximal article of inner rami fringed with 3-4 bifurcate setae.

Pleopods 1–3 rami with 12–15 articles each. *Uropod 1* protopodite with 1 basofacial spine, 4 dorso-lateral spines and 1 dorso-medial spine; exopodite : endopodite length is 1 : 0.9; rami straight with single spines along outer margins; both with 5 strong notched spines apically and sub-apically. *Uropod 2* exopodite as long as endopodite. *Uropod 3* protopodite with two groups of strong notched spines on apex; endopodite 0.6 of protopodite length, with 2 spines and 3–5 long setae apically; exopodite 1.5× longer than protopodite, with 3 groups of lateral spines, long simple setae along inner margin, 5 spines and 10 long setae apically.

Female, 8.5 mm (X43383/Cr-1614-FEFU), sexually dimorphic characters (Figures 7A–I). Body smaller than male, with more slender body. *Antenna 1* 50% of total body length; antenna 2 short reaching 40% length of antenna 1. *Gnathopods 1* and *2* propodi similar, sub-rectangular, palmar margins sub-transverse, short and weakly armed with spines. Carpus of gnathopod 2 flexible, as long as propodus. *Uropod 3* protopodite lacking lateral group of apical spines; exopodite 2× longer than protopodite. *Oostegites 2–5* on gnathopod 2 and pereopods 3–5.

Variability. The pilosity of posterior margin of pereopod 5 bases is very variable, ranging from normally setose to very densely setose. The form of postero-ventral corner and number of spines on ventral margin of epimeral plates varies slightly. Maxilla 1 distal palp article with 5–8 apical setae; outer plate with 105–114 spines; inner plate with 20–24 setae. Endopodite of uropod 3 with 1 or 2 spines on apex. The propodus of gnathopod 2 on female specimens from the Sredne-Shakuranskaya Cave were somewhat elongated, with palmar margin distinctly transverse; rami of uropod 2 of both sexes unarmed along outer margins. One specimen was observed in sample from the "Istočnik Tcebel'da" Cave with the same character of uropod 2 rami.

Remarks. Taxonomy of the genus *Zenkevitchia* is confusing and requires a thorough revision. The first mention of *Z. admirabilis* was given by Birstein in 1940, who gave a description and drawings of a male (14.0 mm body length), but without specifying the geographical origin of the material. However, a year later Birstein (1941) provided a detailed description of the genus as well as the species *Z. admirabilis*, based on a 4.0 mm female from a cave near Andreevka (Apra), not far from Sukhumi. Further, Birstein and Ljovuschkin (1970) provided an expanded description of *Z. admirabilis* and provided information on its distribution within Abkhazia.

A detailed analysis of these species descriptions has left the impression that *Z. admirabilis* is represented by a series of different species in this region. Indirectly, this is evidenced in remarks by Birstein and Ljovuschkin (1970, p. 1473, [here translated from Russian]): "The study of a large specimens (up to 20 mm in length) suggests a significant age-related variation in this species, description of which is done on the basis of a female of 4 mm length and supplemented by a 14 mm male. " ..." with increases in body size, there are changes in the armament of mouthparts, gnathopods and probably the number of spines on urosomal segments". Due to the fact that the 14.0 mm male (Birstein 1940) and 4.0 mm female (Birstein 1941) belongs to a different species (Table 1) and both descriptions are very poor we consider that detailed comparison is premature in this work. The searching of type series from cave near Andreevka was not

Figure 8. Habitat of *Zenkevitchia yakovi* sp. n. in the Sredne-Shakuranskaya cave, South Caucasus. One of the authors (DMP) sampling amphipods in the cave rivulet.

successful, and the cave was mined in 1992–93 during the Georgian-Abkhaz conflict (Turbanov, pers. comm., 2015), therefore, a complete revision of the *Z. admirabilis* species-complex is not yet feasible. Comparison of *Z. yakovi* sp. n. with available de-

scriptions of *Z. admirabilis* did not allow us to identify any significant morphological differences, except for probably unimportant differences in body size and questionable dispersion in outer plate of maxilla 1, bearing ≥ 23–50 spines for *Z. admirabilis* (see Table 1). We cannot excluded the possibility that the relatively small number of spines on the outer plate of maxilla 1 in the original descriptions may be the result of Birstein not using a crushing technique to reveal additional spines. Based on the fact that *Z. yakovi* sp. n. fits within the ranges of morphological variability indicated by Birstein for *Z. admirabilis* (Birstein and Ljovuschkin 1970; Table 1), but genetically both lineages are quite distinct, we feel the hidden diversity within *Zenkevitchia* requires examination of additional material from other localities.

Distribution and ecology

Besides locus typicus of *Z. yakovi* sp. n. at the cave "Istočnik Tcebel'da" this species is known from Sredne-Shakuranskaya Cave, Gulripshi district of Abkhazia. The structure of the mouthparts, particularly unusual structure of outer plate with numerous long multi-toothed spines, suppose its filtering function. Analysis of the esophagus of a few individuals showed only the presence of detritus.

The nature of the morphological variability and divergence of COI gene sequences (pairwise distances 0.005) suggests that ,between the two caves "Istočnik Tcebel'da" and Sredne-Shakuranskaya, two populations of the same species are present.

Acknowledgements

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